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The Effect of Chemical Irritation on the Economic Coefficient of Sugar

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It has been known since Raulin's* account of the nutrition of fungi that certain metallic salts—notably those of zinc—induce a more rapid growth of fungi than is normal, although, as has been shown by more recent work,† he somewhat misinterpreted the action of these salts. It is now well known, as has been demonstrated by many competent experimenters, that a much simpler nutrient solution than was thought necessary in Raulin's time is adequate for an entirely normal development of fungi. With some available source of carbon and nitrogen it is only necessary to add salts containing potassium, magnesium, sulphur, phosphorus, and a trace of iron to provide a suitable substratum for the growth of these saprophytic hyphomycetous fungi which have been experimented with.‡ The action of the metallic salts noted by Raulin, as well as of others not considered by him, is to be regarded as a response to a chemical irritation which in some way hastens the metabolic activity of the fungus. The result is the production within a given time of a greater amount of dry substance as compared with the same fungus grown under similar conditions, but on solutions free from the irritant. For further particulars as to the range of substances which affect this abnormal growth and

* Études Chimique sur la Vegetation, Ann. d. Sc. Nat. Bot. V. **11** : 91. 1869.

† Die Beeinflussung des Wachsthums einiger Pilze durch chemische Reize. Richards, Prings. Jahrb. **30** : 665. 1897.

‡ Pfeffer, Pflanzenphysiologie, **1** : 374.

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the comparative violence of the irritation, reference is made to the paper already cited.* It will be seen that numerous metallic salts and some organic substances were found which more or less markedly bring about the above noted effect and that their action is constant, although the organic substratum, or the nitrogen source, be changed. Iron salts have a double effect, acting in the first place as a necessary substance for the growth of fungi,† and in the second place in stronger solutions having a distinctly irritating effect.

It was the object of the following recorded experiments to endeavor to throw some light on the physiological action of this chemical irritation, to approach a little nearer to discovering the underlying cause of the abnormal growth of these fungi under such conditions. As a first step in this direction cultures and analyses were made to determine if there was any regular and considerable variation in the economic coefficient of the organic food material supplied to the fungus.

Because of its greater ease in determination sugar was employed as the organic basis of the nutrient solution and many analyses were made to determine what relation the weight of dry substance produced for the amount of sugar used bore between the normal culture, and those growing under chemical irritation. It would, no doubt, have been interesting for further comparison to have used other organic substrata, such as glycerin, but it was hardly necessary in this instance to do so in order to prove the point desired, and the difficulty of accurate quantitative determination of glycerin made it impracticable with the facilities at hand for such research.

For the cultures the usual method of growing the fungi in flasks was employed. For most of the experiments the ordinary Florence flasks of about 125 cc. capacity were used; they were selected with due care as regards similarity of shape, and any error due to difference in area of the surface of the culture fluid could not have been considerable. In these flasks 50 cc. of the nutrient solution was used; for larger quantities, where 100 cc. was taken, Erbenmeyer flasks of Jena glass, about 250 cc. in capacity,

* Richards, *l. c.*

† Molisch, Pflanze in ihren Beziehungen zum Eisen. Jena, 1892. Benecke, Die zur Ernährung der Schimmelpilze nothwendige Metalle. Prings. Jahrb. **27**: 487. 1895.

were selected. The sowings of the fungus spores were not made by the addition of water in which the spores hung suspended, since it was desired not to weaken the solutions and thereby involve another chance of error in the subsequent analyses. Instead of this method, small pieces of heavy glass rod (about 8 mm. diam.) were taken, their ends slightly moistened and then rubbed on the dry stock culture of the desired fungus. The bits of glass rod, with the attached spores, were then dropped in the prepared flasks; a slight shaking served to dislodge the spores which promptly rose to the surface and with sufficiently even distribution to insure an even growth of the fungus when they germinated. In this way the cultures were provided with at least an approximately equal number of spores, certainly above the maximum required to produce an unbroken carpet of mycelium, and that, as has already been shown, is sufficient to make an equal growth on surfaces of the same area.

As in the previous investigations the greatest care was taken to have all of the substances used for the culture fluids of the greatest practicable degree of purity. The chemically pure salts prepared by Merck & Co. were used and again recrystallized. The sugar was of the best quality obtainable in the market and showed on many tests to be free from impurities. The water was twice distilled, once over a tin-lined still and the second time over glass with alkaline permanganate. It should be added that due care was taken that none of the permanganate passed over. By all tests employed as well as by the evidence of the experiments themselves, the water was shown to be pure. For the irritant substances, the zinc sulphate and lithium carbonate, from which the chloride was prepared, were kindly given the writer by Professor T. W. Richards, of Harvard University. Of the other salts the nickel sulphate and the ferric chloride were the purest obtainable and further purified by successive recrystallizations.

Only one nutrient solution was used—that recommended by Pfeffer * which is identical with solution *A* of the writer's previous paper.† The formula is as follows:

* Election organischer Nährstoffe. Prings. Jahrb. **27**: 238. 1895. Pflanzenphysiologie, **1**: 375.

† Richards, *l. c.* p. 667.

NH_4NO_3	1.00
KH_2PO_4	.50
$MgSO_4$	.25
Sugar	5.00
Water	100.00
Trace of iron	

The fungi experimented with were *Sterigmatocystis* (*Aspergillus*) *nigra*, *Penicillium glaucum* and *Trichothecium roseum*.

In making up the solutions it was found most convenient as well as most accurate to prepare them in considerable quantities with all the ingredients except the sugar and of exactly twice the strength desired. Of the solutions thus prepared and with their respective amounts of the irritant substances added, 25 or 50 cc. were taken and exactly the same amount of an accurately prepared 10% sugar solution added. In all of the processes the same pipettes were used throughout and were handled in the same manner, great care being taken of course not to contaminate one solution with another, particularly the control solutions. In this manner it was found practicable to prepare quickly solutions containing a standard of 5% sugar with all the accuracy needed for this work. Numerous test analyses were made of solutions made up after this manner and it was found that they did not vary more than 0.005 grm. in sugar content. It is obvious that it was needful to have confidence in the accuracy of the solutions for upon this point depended the entire result of the work.

When the crop of fungus was harvested the flasks were well shaken and the contents filtered. To the filtrate 1 cc. of a 5 % solution of HCl was added and time being allowed for the inversion of the sugar the HCl was then neutralized with Na_2CO_3 and a sufficient amount of water added to dilute the solution to just twice its original bulk, thus weakening it sufficiently to allow of an accurate analysis. From these solutions always two and sometimes more analyses were made. The control cultures were usually two in number; the average between them being the figures printed in the tables. The determination of the sugar was made by the Fehling method. For this purpose the usual solutions of $CuSO_4$ and of alkaline Rochelle salts were made up and mixed freshly for each set of analyses. The Fehling solution was tested against a

standard sugar solution each time. The factor (Allen's Industrial Chemistry, vol. I., p. 226) of 10 cc. Fehling solution = 0.0475 cane sugar after inversion was used as the basis of all calculations.

The dry weight of fungus was determined in the usual way, the crop having been collected on a weighed filter was dried in an oven at the temperature of about 70° C. to constant weight.

In the absence of thermostat the cultures were grown at the ordinary room temperature in the laboratory or at a somewhat higher temperature in a room which served also as a conservatory. The cultures were consequently subjected to some fluctuation of temperature, possibly somewhat to the disadvantage of the results obtained. It would, undoubtedly, have been preferable to have grown the *Sterigmatocystis* cultures at a point nearer the optimum for that fungus between 30° and 34° C. In the case of the *Penicillium* the room temperature approximated more nearly the lower optimum of that fungus. In spite of the variations, however, the results for both correspond satisfactorily, the *Sterigmatocystis* cultures being allowed to grow for a somewhat longer period than would otherwise have been necessary.

It would, indeed, have been well to have determined the respiration quotient in relation both to the increased growth and the economic coefficient but the writer was unable at the time to do so, although, it is his intention to experiment in this line in the future. The facts demonstrated, however, show much as to the economic coefficient of the sugar in relation to the abnormal growth caused by chemical irritation despite the fact that at present they cannot be compared with the CO_2 coefficient.

It will be seen by comparison with the results of Kunstmann* that the averages of the economic coefficient obtained from the control cultures is correct. This average approximates 2.00 for the ratio between the amount of sugar used for the dry weight of fungus produced or, as may better be expressed, 0.50 grm. of dry substance for each gram of sugar consumed. In table I. of Kunstmann's paper the average coefficient for those cultures grown between the temperatures of 17° and 25°C. is 2.05 = 0.49. This serves as a check for the results recorded herein.

* Ueber das Verhältniss zwischen Pilzernte u. verbrauchter Nährung. Inaug. Dissert., Leipzig, 1895.

Taking the control cultures as a base from which comparison can be made, those cultures to which an irritant substance was added now demanded attention. From the results previously obtained such degrees of concentration as showed a marked irritant were employed, the stronger solutions where in the case of the poisonous salts a secondary toxic effect was noted, were not employed, except in one series. With this last named exception all of the ZnSO4 series comprised the following percentages of the anhydrous salt 0.002 %; 0.004 %; 0.008 %; 0.032 %; the last named concentration being just within the range of the toxic effect. In those cultures to which iron salts were added, a much greater degree of concentration is indicated, for it will be remembered that iron has a double effect, first as a necessary food substance for the fungus, and secondarily, when present in larger quantities as an irritant. Consequently the percentages of Fe_2Cl_6 were 0.05 %; 0.10 %; 0.20 %. In the same way the lithium salt, in this case lithium chloride, although not an indispensable ash constituent is not effective as an irritant, except in comparatively strong solutions, and apparently does not exert any poisonous influence on these hyphomycetous fungi. It was used in the following concentrations of 0.125 %; 0.350 %; 0.375 %. In the few series with nickel salts the sulphate was not used in concentrations very much greater than with the ZnSO4, for like the latter salt it is ultimately a poison. The citations above given are in fractions of a per cent., for the sake of comparison with the writer's previous paper which has already been referred to, but it will be observed that in the tables the equivalents of the solutions are given in fractions of the *normal* solution. This method of reckoning in gram-atoms of the irritant or toxic base was employed by Kahlenberg and True* and affords a much better standard for comparison for future works in this line than expressions in terms of per cent. Since in every salt used in these experiments herein described the acid may be regarded as entirely neutral in its effect on the growth of the fungi the whole of the irritant effect is to be referred to the base of the particular salt employed.

Upon examination of the tables it will be seen that the curve of the economic coefficient of the sugar rises with the increase in

* Bot. Gaz. **22**: 81–124. 1896.

the dry weight of the fungus, but more abruptly. For instance, in the case of the cultures with $ZnSO_4$ as in the case of irritation it attains its maximum of 0.58 (see general averages, Table V.) at a concentration of 0.004% of this salt, at which point also the maximum weight is shown. In solutions of greater strength the coefficient falls off but more rapidly than does the abnormal growth. At a concentration of 0.032% when the toxic effect of the $ZnSO_4$ begins to be noticeable the economic coefficient has fallen almost to that of the control as also has the weight of dry substance. In yet stronger solutions in which the growth is much retarded the amount of sugar used remains, however, in about this same relation. The cultures in which Fe_2Cl_6 was used do not show the same regularity. Up to the strength of 0.1% the rise of the economic coefficient of the sugar from 0.46 to 0.56 keeps pace with the increase of weight from about 330 mg. to 800 mg. and agrees with the results found with the $ZnSO_4$, but beyond that at a concentration of 0.2% the average of the economic coefficients falls somewhat while the average weight of the fungus crop increases. This was more apparent in the *Penicillium* cultures than in those with *Sterigmatocystis*. In the latter both the weight and ratio remain about equal, while in the *Penicillium* only one series shows any increase (XVIII.) and series XX. indicates a distinct falling off of the coefficient although a considerable gain in weight is shown in the 0.2% culture over that with but 0.1% of Fe_2Cl_6. It is to be observed, however, that in this case the ratio of 0.82 given for the 0.1% culture stands alone in being the highest found in any series. It is this series that has so materially affected the averages, but since no legitimate reason could be discovered for throwing it out it was necessarily included with the rest. In the series with LiCl the two with *Sterigmatocystis* show the same peculiarity, for in the stronger concentration of 0.375% there is a distinct gain in weight with some falling off in the availability of the sugar consumed (series XXII., XXIII.). In the *Penicillium* cultures the ratio rises even in the strongest solution employed but at 0.375% the gain does not correspond to the increase in weight over the 0.25% concentration. In the *Trichothecium* series there is no marked change. All of the series with LiCl agree, however, in showing in the weaker solutions of 0.125% and 0.25% an increase of the

ratio over that found in the control cultures corresponding to that found in other experiments. A few series were tried with a nickel salt, the results falling in line with those obtained with the $ZnSO_4$ cultures, the curve of the economic coefficient of the sugar following a course similar to that of the gain in weight.

In order to compare the abnormal growth caused by these inorganic salts with that produced by organic substances a couple of series were carried through with cocaine as an irritant. As is shown in the previous paper these fungi do not respond very violently to the organic substances therein mentioned and cocaine was selected as being the most potent. The results were surprisingly definite; as will be seen a distinct increase of weight resulted with also an appreciable gain in the ratio amounting to about 0.04.

It will be seen that, although the effectiveness of sugar as a source of organic nutrition increases in general with the increase of growth induced by the irritant substance and diminishes as the latter diminishes, the economic coefficient does not exactly parallel in its curve the gain of dry substance. For instance, supposing that the dry weight of a control culture be 1 and the economic coefficient of the sugar be 0.50, although the dry weight of a culture under similar conditions but with the addition of an irritant be 2 the economic coefficient is not 1.00 but much lower on the average, say 0.60. Indeed, it is not to be expected that the economic coefficient should vary in the same proportion as the increase of weight. Such an example as given above—the ratio of weights is often higher as much as 1 to 3—would require that *all* of the sugar used be available for the production of the fungus mycelium, an impossibility in any event since such a condition would preclude the respiration of any CO_2. Nor is it necessary that the available portion of the sugar used increase in a similar proportion to the dry substance, for it will be remembered that the effect of the irritant substance must, as long as the food supply is not greatly exhausted, be cumulative. Even a smaller increase of the economic coefficient of the sugar than that absolutely found would serve to account for a considerable increase in weight. It is evident from the experiments that of the sugar used more is actually available for the fungus and that provides for and implies a more rapid growth of the latter. Granting this together with the accompanying

necessity that the irritant substance is acting continually, it is easy to understand that any gain in weight might be indefinitely multiplied as long as the food supply was sufficient. Since, up to a certain point, the economic coefficient of the sugar rises with the increase of dry weight shows that there must be some relation between the two, that the latter phenomenon must in some measure at least be dependent upon the former. The actual gain in the economic coefficient must at any one time be very small and it is highly probable that given time any two cultures, the one with and the other without an irritant substance added would tend to become equalized.

As the weight of the crop falls with the increase of $ZnSO_2$ so also does the economic coefficient diminish, but the writer would not be prepared to maintain that the toxic effect of this substance is in itself merely the diminishing of the economic coefficient to a vanishing point. It is not to be supposed that the irritant salt acts directly on the sugar but on the fungus in which no doubt other and more subtle changes in the protoplasm are brought about. As was shown indeed in one series even in very much stronger solutions of $ZnSO_4$ where the growth is materially diminished by the salt, the economic coefficient remains practically the same as in the normal. A further discussion of the *toxic* action of this salt is, however, not within the limits of this paper.

While it would be manifestly improper with evidence afforded by only a comparatively few series of experiments from but a single point of view to theorize too widely as to the nature of this chemical irritation, the writer feels justified in arriving at the conclusion that the increase in the availability of the sugar consumed is at least one factor and an important one in determining the increase of growth. In just what way the irritant influences the metabolic activity of the fungus hyphae must be at present at least merely a matter of speculation. The irritant substance is not in itself a source from which energy is available.

In their action as poisons the salts of zinc, nickel, manganese and lithium would come under the third group of poisons as recognized by Loew in his "Naturliche System der Gift Wirkungen,"* which includes those bases that by their power of forming salts with the protein substances of the protoplasm induce disturbances

* Munich, 1893. See also Davenport, Experimental Morphology, 1 : 12.

which ultimately end in death. It may be that such indeed is the case, but the poisonous substances so formed being in such minute quantities, owing to the dilute solutions used, do not serve to kill the protoplasm, but merely stimulate its molecular activity in an endeavor to throw off the irritant substance, or to induce what might be called a secondary katalytic action. The results with the salts of iron which are not poisonous do not, however, uphold such a view, yet it is not impossible that in stronger solutions the apparently inocuous base, iron, might prove to exert a poisonous influence. This might be impossible to demonstrate, since the necessary concentration to produce any deleterious effect would be so great as to confuse the results with the osmotic action of the solution. If it is not possible to admit any such semi-toxic action on the part of the irritant substances, it is necessary to fall back upon the idea of their action being strictly katalytic, as suggested by Pfeffer,* or simply to include the phenomenon under the comprehensive phrase "physiological counter-reaction."

The results of these experiments may be briefly stated as follows:

That the direct action of irritant substances (in this case inorganic salts), which produce an increased growth of certain fungi is to enable the latter to dispose more economically of the sugar used (*i. e.*, to raise the economic coefficient of the sugar) thereby permitting a more rapid production of dry substance in a given time.

That the increase of the economic coefficient is not in proportion to the percentage increase in weight.

That the economic coefficient again decreases when in poisonous substances the maximum of growth is passed, but that it apparently does not ever fall much below the normal.

This work was begun in the Cryptogamic Laboratory of Harvard University in 1897–98 and completed at Barnard College, New York, in 1898–99. The writer would here express his thanks to Professor H. B. Hill, Director of the Chemical Laboratory of Harvard University, for his courtesy in allowing the use of the facilities of that laboratory.

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* Pfeffer, Prings. Jahrb. **28**: 238. 1895.

TABLE I.

In all of the cultures a standard of 5% sugar was used. The series are indicated in Roman numerals.

I. *Sterigmatocystis nigra.* $ZnSO_4$ added. Grown at room temp. about 20°C. Harvested one week after sowing.

% $ZnSO_4$. Fraction normal $ZnSO_4$.	Control.	.002% .000125	.004% .00025	.008% .0005	.032% .002
I.					
Weight crop mg.	535	846	1020	945	
Sugar used mg.	930	1220	1400	1315	
Sugar residue mg.	1570	1280	1100	1185	
Econ. Coeff. Fungus : sugar.	1.74	1.44	1.37	1.39	
Econ. Coeff. Sugar : fungus.	0.57	0.69	0.73	0.72	
II as in I. Temp. about 20°C. 7 days.					
Weight crop mg.	640	912	1104	842	800
Sugar residue mg.	1845	1315	1300	1370	1305
Sugar used mg.	955	1185	1200	1130	1195
Econ. Coeff. Fungus : sugar.	1.49	1.29	1.09	1.34	1 50
Econ. Coeff. Sugar : fungus.	0.67	0.77	0.92	0.75	0.67
III as in I. Temp. about 20°C. 6 days.					
Weight crop mg.	330	535	817	800	400
Sugar residue mg.	1825	1605	1320	1284	——
Sugar used mg.	675	895	1180	1216	——
Econ. Coeff. Fungus : sugar.	2.00	1.67	1.44	1.52	——
Econ. Coeff. Sugar : fungus.	0.49	0 59	0.69	0.66	——
IV as in I. Temp. circa 24°C. 8 days.					
Weight crop mg.	510	830	943	912	615
Sugar residue mg.	1306	1164	1029	1032	1461
Sugar used mg.	1194	1336	1471	1468	1039
Econ. Coeff. Fungus : sugar.	1.95	1.73	1.56	1.61	1.69
Econ. Coeff. Sugar : fungus	0.51	0.59	0.64	0.62	0.59
V as in I. Temp. circa 24°C. 5 days.					
Weight crop mg.	305	523	610	600	520
Sugar residue mg.	1912	1637	1536	1540	1164
Sugar used mg.	588	863	964	960	925
Econ. Coeff. Fungus : sugar.	1.83	1.65	1.58	1.60	1.74
Econ. Coeff. Sugar : fungus.	0 55	0.60	0.64	0.62	0.59
VI as in I. Temp. circa 20°C. 7 days.					
Weight crop mg.	308	527	622	580	489
Sugar residue mg.	1980	1620	1480	1525	1705
Sugar used mg.	620	880	1020	975	895
Econ. Coeff. Fungus : sugar.	2.01	1.67	1.64	1.68	1 83
Econ. Coeff. Sugar : fungus.	0.50	0.61	0.60	0.62	0.55

TABLE II.

VII. *Penicillium glaucum*. $ZnSO_4$ added. Grown at room temperature. Harvested nine days after sowing. 100 cc. culture fluid in flasks 200 cc. capacity.

% $ZnSO_4$. Fraction normal $ZnSO_4$.	Control.	.002 % .000125	.004% .00025	.008% .0005	.032% .0020
VII.					
Weight crop mg.	430	613	940	836	503
Sugar left mg.	4093	3809	3130	3480	3500
Sugar used mg.	997	1191	1870	1520	1500
Econ. Coeff. Fungus : sugar.	2.11	1.96	1.99	1.81	2.30
Econ. Coeff. Sugar : fungus.	0.46	0.51	0.50	0.55	0.46
VIII as in VII. Temp. circa 20°C. 9 days.					
Weight crop mg.	395	705	693	674	581
Sugar residue mg.	4048	3393	3409	3403	3559
Sugar used mg.	952	1607	1591	1597	1441
Econ Coeff. Fungus : sugar.	2.41	2 28	2.31	2.37	2.48
Econ Coeff. Sugar : fungus.	0.41	0.44	0.44	0.44	0.41
IX as in VII. Temp. circa 20°C. 8 days.					
Weight crop mg.	295	416	683	618	
Sugar residue mg.	4426	4322	3873	3937	
Sugar used mg.	574	678	1127	1063	
Econ. Coeff. Fungus : sugar.	1.95	1.70	1.65	1.72	
Econ. Coeff. Sugar : fungus.	0.51	0.59	0.61	0.58	
X as in VII. Temp. circa 20°C. 8 days.					
Weight crop mg.	362	700	910	685	525
Sugar left mg.	4184	3710	3453	3550	3793
Sugar used mg.	816	1290	1547	1450	1207
Econ. Coeff. Fungus : sugar.	2.26	1.85	1.70	2.12	2.30
Econ. Coeff. Sugar : fungus.	0.43	0.54	0.59	0.47	0.44
50 cc. XI Conditions as in VII. *Trichothecium roseum*. Harvested 10 days after sowing. Temp. circa 22°C.	*Trichothecium roseum.*				
Weight crop mg.	112	203	203	196	091
Sugar residue mg.	2231	2052	2064	2013	2278
Sugar used mg.	269	448	436	447	222
Econ. Coeff. Fungus : sugar.	2.40	2.21	2.15	2.28	2.44
Econ. Coeff. Sugar : fungus.	0.41	0.45	0.48	0.44	0.41
XII as in XI. *Trichothecium roseum*. Temp. circa 22°C.					
Weight crop mg.	95	205	183	194	104
Sugar residue mg.	2298	2098	2152	2126	2281
Sugar used mg.	202	402	348	374	219
Econ. Coeff. Fungus : sugar.	2.13	1.99	1.90	1.93	2.11
Econ. Coeff. Sugar : fungus.	0.48	0.51	0.53	0.52	0.46

TABLE III.

XIII. *Sterigmatocystis nigra.* Excess of Fe_2Cl_6 added. Temperature about 25°C. Harvested seven days after sowing. Culture flasks 125 cc. 50 cc. culture fluid.

% Fe_2Cl_6 Fraction normal Fe_2Cl_6.	Control.	0.050 % .00015	0.100 % .0003	0.200 % .0006
XIII.				
Weight crop mg.	320	515	710	700
Sugar residue mg.	1796	1516	1187	1205
Sugar used mg.	704	984	1313	1295
Econ. { Fungus : sugar.	2.20	1.91	1.85	1.85
Coeff. { Sugar : fungus.	0.45	0.52	0.54	0.54
XIV as in XIII. Temp. circa 23°C. 6 days.				
Weight crop mg.	285	491	683	694
Sugar residue mg.	1944	1616	1346	1369
Sugar used mg.	556	884	1154	1131
Econ. } Fungus : sugar.	1 95	1.80	1.69	1.63
Coeff. } Sugar : fungus.	0.51	0.55	0.60	0.62
XV as in XIII. Temp. circa 33°C. 6 days.				
Weight crop mg.	305	500	690	725
Sugar residue mg.	1890	1595	1355	1268
Sugar used mg.	610	905	1145	1232
Econ. { Fungus : sugar.	2.00	1.81	1.66	1.70
Coeff. { Sugar : fungus.	0.50	0.55	0.61	0.59
XVI as in XIII, but with 100 cc. Temp. circa 24°C. 6 days.				
Weight crop mg.	711	1240	1635	1610
Sugar residue mg.	3364	2446	2028	1796
Sugar used mg.	1636	2554	2972	3204
Econ. { Fungus : sugar.	2.31	2.06	1.94	1.99
Coeff. { Sugar : fungus.	0.46	0.49	0.52	0.50

TABLE IV.

XVII. *Penicillium glaucum*. Excess of Fe_2Cl_6 added. Temp. about 20°C. Harvested nine days after sowing. Culture flasks 125 cc. 50 cc. culture fluid.

% Fe_2Cl_6. Fraction normal Fe_2Cl_6.	Control.	0.051 % .00015	0.10% .0003	0.20% .0006
XVII.				
Weight crop mg.	160	300	410	390
Sugar residue mg.	2108	1837	1627	1650
Sugar residue mg.	392	663	873	850
Econ. } Fungus : sugar.	2.45	2.21	2.13	2.18
Coeff. } Sugar : fungus.	0.41	0.45	0.47	0 45
XVIII *Penicillium* as in XVII Temp. circa 21°C. 7 days.				
Weight crop mg.	148	273	386	420
Sugar residue mg.	2158	1930	1672	1647
Sugar used mg.	342	570	818	853
Econ. } Fungus : sugar.	2.31	3.09	2.12	2.03
Coeff. } Sugar : fungus.	0.43	0.46	0.46	0.49
XIX *Penicillium* as in XVI 100 cc. Temp. circa 19°C. 8 days.				
Weight crop mg.	375	830	1.012	.981
Sugar residue mg.	4128	3324	3148	3125
Sugar used mg.	872	1676	1852	1875
Econ. } Fungus : sugar.	2.30	2.02	1.83	1.91
Coeff. } Sugar : fungus.	0.43	0.49	0.54	0.52
XX *Penicillium* as in XIX 100 cc. Temp. circa 19°C. 10 days.				
Weight crop mg.	546	945	1.270	1.348
Sugar residue mg.	3890	3265	3450	2816
Sugar used mg.	1110	1735	1550	2184
Econ. } Fungus : sugar.	2.03	1.84	1.22	1.62
Coeff. } Sugar : fungus.	0.49	0.54	0.82	0.62
XXI *Penicillium* as in XIX 100 cc. Temp. circa 19°C. 9 days.				
Weight crop mg.	280	463	617	680
Sugar residue mg.	4385	4074	3891	3810
Sugar used mg.	615	926	1109	1190
Econ. } Fungus : sugar.	2.16	2.00	1.71	1.75
Coeff. } Sugar : fungus.	0.46	0.50	0.58	0.58

TABLE V.

Average of $ZnSO_4$ Cultures.

% $ZnSO_4$. Fraction Normal $ZnSO_4$.	Control.	.002% .000125	.004% .00025	.008% .0005	.032% .002
Sterigmatocystis, 6 series.					
Average weight crop mg.	438	695	853	780	471
Econ. Coeff. Fungus : sugar.	1.91	1.65	1.45	1.47	1.76
Econ. Coeff. Sugar : fungus.	0.52	0.60	0.69	0.68	0.57
Penicillium, 4 series.					
Average weight crop mg.	370	650	807	707	402
Econ. Coeff. Fungus : sugar.	2 20	1.98	1.85	1.90	2.35
Econ. Coeff. Sugar : fungus.	0.45	0.52	0.54	0.53	0.44
Av. Econ. Coeff, both fungi.					
Fungus : sugar.	2.05	1.83	1.65	1.73	2.00
Sugar : fungus.	0.48	0.56	0.62	0.60	0.50

Average of Fe_2Cl_6 Cultures.

% Fe_2Cl_6. Fraction Normal Fe_2Cl_6.	Control.	.050% .00015	.10% .0003	.20% .0006
Sterigmatocystis, 4 series.				
Average weight crop mg.	316	532	725	731
Econ. Coeff. Fungus : sugar.	2.11	1.89	1.78	1.79
Econ. Coeff. Sugar : fungus.	0.48	0.52	0.56	0.56
Penicillium, 5 series.				
Average weight crop mg.	354	660	881	906
Econ. Coeff. Fungus : sugar.	2.25	2.07	1.80	1.90
Econ. Coeff. Sugar : fungus.	0.44	0.48	0.55	0.52
Av. Econ. Coeff., both fungi.				
Fungus : sugar.	2.18	1.98	1.79	1.85
Sugar : fungus.	0.46	0.50	0.56	0.54

TABLE VI.

Series with LiCl added. XXII., XXIII., *Sterigmatocystis;* XXIV.-XXVI., *Penicillium;* XXVI., *Trichothecium.* All in 125 cc. flasks with 50 cc. culture fluid.

% LiCl. Fraction normal LiCl.	Control.	0.125 % .03	0.250 % .06	0.375 % .09
XXII. *Sterigmatocystis.*				
Temp. circa 24°C. 8 days.				
Weight crop mg.	295	435	420	681
Sugar left mg.	1778	1539	1580	1090
Sugar used mg.	722	961	920	1410
Econ. Coeff. Fungus : sugar.	2.45	2.21	2.19	2.07
Econ. Coeff. Sugar : fungus.	0.41	0.45	0.45	0.49
XXIII. *Sterigmatocystis.*				
Temp. circa 23°C. 6 days.				
Weight crop mg.	315	451	532	610
Sugar left mg.	1892	1733	1617	1548
Sugar used mg.	608	767	833	952
Econ. Coeff. Fungus : sugar.	1.93	1.70	1.66	1.56
Econ. Coeff. Sugar : fungus.	0.52	0.59	0.60	0.64
XXIV. *Penicillium.*				
Temp. circa 18°C. 9 days.				
Weight crop mg.	180	290	301	466
Sugar residue mg.	2115	1940	1964	1699
Sugar used mg.	385	560	536	801
Econ. Coeff. Fungus : sugar.	2.15	1.93	1.78	1.72
Econ. Coeff. Sugar : fungus.	0.48	0.52	0.56	0.58
XXV. *Penicillium.*				
Temp. circa 20°C. 7 days.				
Weight crop mg.	135	242	288	501
Sugar residue mg.	2224	2085	2031	1712
Sugar used mg.	256	414	469	787
Econ. Coeff. Fungus : sugar.	1.90	1.71	1.63	1.57
Econ. Coeff. Sugar : fungus.	0.52	0.59	0.60	0.61
XXVI. *Penicillium.*				
Temp. circa 19°C. 9 days.				
Weight crop mg.	121	264	303	285
Sugar residue mg.	2231	1964	1924	1950
Sugar used mg.	269	536	576	550
Econ. Coeff. Fungus : sugar.	2.22	2.03	1.90	1.93
Econ. Coeff. Sugar : fungus.	0.45	0.49	0.53	0.52
XXVII. *Trichothecium roseum*				
Temp. circa 20°C. 8 days.				
Weight crop mg.	101	195	276	344
Sugar residue mg.	2286	2143	1986	1877
Sugar used.	214	357	513	623
Econ. Coeff. Fungus : sugar.	2.12	1.94	1.86	1.81
Econ. Coeff. Sugar : fungus.	0.47	0.52	0.54	0.55

TABLE VII.

Sterigmatocystis nigra. XXVIII.–XXX. with $NiSO_4$. XXXI. and XXXII. with Cocaine. Otherwise as in previous cultures. 50 cc. culture fluid.

% $NiSO_4$ Fraction Normal $NiSO_4$.	Control.	0.008 % .0005	0.016 % .001	0.033 % .002
XXVIII Temp. circa 24°C. 7 days				
Weight crop mg.	250	360	885	210
Sugar residue mg.	2043	1916	1156	2099
Sugar used mg.	457	584	1344	401
Econ. } Fungus : sugar.	1.83	1.60	1.52	1.91
Coeff. } Sugar : fungus.	0.55	0.62	0.67	0.53
XXIX as in XXVIII. Temp. circa 24°. 7 days.				
Weight crop mg.	231	365	430	215
Sugar residue mg.	1969	1740	1714	2010
Sugar used mg.	530	760	786	490
Econ. } Fungus : sugar.	2.30	2.14	1.92	2.28
Coeff. } Sugar : fungus.	0.44	0.48	0.53	0.44
XXX as in XXVIII. Temp. circa 23°. 7 days.				
Weight crop mg.	305	482	595	300
Sugar residue mg.	1835	1555	1455	1860
Sugar used mg.	665	945	1045	640
Econ } Fungus : sugar.	2.18	1.95	1.76	2.13
Coeff. } Sugar : fungus.	0.46	0.51	0.57	0.47
XXXI Cocaine, otherwise as in XXVIII.	Control.	0.1 %	0.2 %	
Weight crop mg.	360	520	600	
Sugar residue mg.	1658	1382	1192	
Sugar used mg.	842	1118	1308	
Econ. } Fungus : sugar.	2.34	2.15	2.18	
Coeff. } Sugar : fungus.	0.42	0.48	0.46	
XXXII Cocaine, otherwise as in XXVIII.				
Weight crop mg.	275	510	591	
Sugar residue mg.	1929	1557	1616	
Sugar used mg.	572	943	884	
Econ. } Fungus : sugar.	2.08	1.85	1.80	
Coeff. } Sugar : fungus.	0.47	0.54	0.55	